Immunologic Abnormalities in Humans Exposed to Chlorpyrifos: Preliminary Observations

JACK D. THRASHER
Thrasher & Associates
Northridge, California
ROBERTA MADISON
Department of Health Science
California State University
Northridge, California
ALAN BROUGHTON
Antibody Assay Laboratories
Santa Ana, California

ABSTRACT. Twelve individuals who were exposed to chlorpyrifos were studied 1–4.5 y following exposure to determine changes in the peripheral immune system. The subjects were found to have a high rate of atopy and antibiotic sensitivities, elevated CD26 cells ($p < .01$), and a higher rate of autoimmunity, compared with two control groups. Autoantibodies were directed toward smooth muscle, parietal cell, brush border, thyroid gland, myelin, and ANA. Chlorpyrifos exposure was implicated in the immunologic abnormalities reported. The immunologic changes were similar to those reported for other pesticides.

INSECTICIDE EXPOSURE is increasingly implicated in peripheral blood immunologic abnormalities in humans. For example, pentachlorophenol (PCP)-exposed subjects have activated T cells (i.e., CD25, CD26), autoimmunity, function immunosuppression, and B cell dysregulation.[1] Exposure to chlordane/heptachlor leads to T cell activation (CD26), increased cortical thymocytes (CD1), decreased frequency of suppressor/inducer phenotype (CD45RA/T4), lowered mitogen response, and autoimmunity.[2,3] Some individuals, following dioxin exposure (a contaminant of chlorinated pesticides), have a depressed delayed-type hypersensitivity reaction, decreased T4 and T11 cells, and a suspected increased in cortical thymocytes.[4] Moreover, women who are exposed to aldicarb in drinking water have an elevated Candida antigen stimulation reaction, increased T8 cells, and a decreased T4/T8 ratio.[5]

In this paper, we present preliminary observations on changes in peripheral blood immunologic phenotype frequencies and report on the presence of autoantibodies after exposure to chlorpyrifos.

Materials and methods

Patients and controls. Twelve patients (4 males and 8 females, 34 ± 8 y of age) were referred to the laboratory by their physicians for immunologic testing because of chronic health complaints that were unrelated to previous health histories (Table 1). Pesticide exposures were confirmed through physician records, subject interviews, material safety data sheets, and laboratory analyses. The exposures were as follows: toxic spill ($n = 1$), office ($n = 2$), bus ($n = 1$), classroom ($n = 1$), and home ($n = 7$). Except for the spill, all insecticides were applied

Table 1.—Summary of Characteristics of 12 Patients Exposed to Chlorpyrifos

Occupation	Age/sex	History of atopy/drug sensitivity*	Type of exposure	Period elapsed from exposure to tests (y)	Additional pesticide exposure
Ranch hand§	34/M	1, 6	Spill	4	Baygon
Musician	38/M	1, 3, 5	Home	1	—
Housewife†	28/F	1, 5, 7	Home	1	—
Housewife‡	29/F	1, 5	Home	4.5	—
Retail owner//	45/F	1, 4	Office	4	—
Teacher†//	46/F	1, 5	Classroom	3.5	—
Engineer‡§	44/M	1, 5	Office	1	—
Housewife	41/F	5	Home	4	—
Housewife‡§//	?/F	None, 5	Home	2	—
Housewife	?/F	Unknown	Home	Unknown	Unknown
Housewife	?/F	Unknown	Home	Unknown	Unknown
Bus driver‡§	39/M	1, 5	Bus	2	Baygon

*1 = hay fever, 2 = childhood asthma, 3 = molds, 4 = bronchitis, 5 = penicillin, 6 = tetracycline, and 7 = sulfa drugs
†Diagnosis by treating physician of either Lupus-like syndrome or SLE.
‡Penicillin sensitivity developed after exposure.
§Developed multiple sensitivities after exposure.
//Antimicrosomal (peroxidase) antibodies.

by licensed operators. Measurements of pesticide residues were not made, except in two cases. Surface wipes at 5 mo post application showed residue levels of chlorpyrifos from < 10 to 2 900 ppm.

Two groups of controls were used. Group A consisted of 28 student volunteers (15 males and 16 females, average age 29 ± 9 y) who were exposed to formaldehyde as described previously.[7] Group B consisted of 29 healthy home dwellers (13 males and 16 females,average age 54 ± 19 y), also as described previously.[8] The atopy history (hay fever, asthma) of Group A was unknown, whereas that of Group B was as reported previously.

Blood collection. Venous blood samples were drawn from the individuals during the 1–4.5 y that followed exposure. All samples, including those obtained from controls, were collected under the supervision of an attending physician in siliconetreated sodium heparinized glass evac-tubes. The blood samples were transported to the laboratory and were used within 24 h with a cell viability of 90% or greater (trypan blue exclusion). All samples were assigned a computer-generated accession number. Quality assurance was performed by positive and negative controls, which were run simultaneously with the unknown samples (autoimmunity).

Lymphocyte markers. The total peripheral white blood cell (WBC) and lymphocyte counts were performed with a Coulter T-540 counter (Coulter, FL). Lymphocyte marker procedures are described elsewhere.[7,9] In brief, peripheral mononuclear cells were isolated by Ficoll Hypaque density gradient.[10] The percentages and absolute numbers (ABS) of lymphocyte subsets per centimeter of blood were enumerated by fluorescent microscopy, utilizing antibodies to surface markers as follows: CD5 (LEU1, T cells); CD4 (LEU3A, T helper cells), CD8 (LEU2A, T suppressor cells); CD19 (LEU10, B cells [Beckton-Dickinson, Los Angeles, CA]); CD25 (IL2 receptor positive cells); and CD25 (TA1 positive cells [Coulter, FL]). All surface markers, except CD26, were identified by indirect immunofluorescence. The CD26 cells were determined by a direct immunofluorescent method.[11,12]

Autoantibody determinations. Antismooth muscle (ASM), antiparietal cell (APC), antibrush boarder (ABB), antimitochondrial (AMIT), and antinuclear (ANA) antibodies were detected by an indirect immunofluorescent method and were expressed as positive at a titer of 1:20.[13]

Antimyelin antibodies were determined by an indirect immunofluorescent method, and rabbit sciatic nerve replaced cervical cord.[14] Titers were expressed positive at 1:8.

Autoantibodies to thyroglobulin and thyroid peroxidase (microsomal) were determined by the method of Beever et al.[15] and distributed by Kronus (San Juan Capistrano, CA). One subject had an ELISA procedure performed (Immunosciences Lab, Los Angeles, CA).

Statistical methods. Analysis of variance was performed to test the hypothesis that there was no difference between the mean percentages and mean ABS of the groups for each cell type. The odds ratios at 95% confidence intervals for the percentage autoantibodies (ASM, APC, AMIT, ABB, and ANA) were estimated, using the Scheffe method for simultaneous construction of 95% confidence intervals for two or more comparison groups.[16]

Results

Health complaints, atopy, and smoking histories. The exposed subjects expressed multiple-organ symptoms for which they sought medical attention. These included an initial flu-like illness followed by chronic complaints of fatigue; central nervous system problems (headaches, dizziness, loss of memory); upper and lower respiratory symptoms; joint and muscle pain; and gastrointestinal disturb-

ances. In addition, individuals who were in environments that contained low concentrations of chemicals—particularly chlorpyrifos and other organophosphorus insecticides—experienced symptoms. Treating physicians reported that the chronic health problems were unrelated to the patients' atopy.

Of the 10 individuals for whom health histories were obtained, the following was found: 8 had seasonal hay fever, 1 had asthma as a child, 1 had childhood bronchitis, 1 had mold sensitivities, 1 (a smoker) had bronchitis, and 5 had sensitivities to antibiotics. One individual developed multiple allergies and 4 developed antibiotic sensitivities after exposure. After the exposure, two of the females were diagnosed with SLE (teacher) and a Lupus-like syndrome by treating physicians (Table 1).

Peripheral blood cell counts. The mean ABS was elevated for WBC, total lymphocytes, CD5, CD4, CD8, and CD26 cells, compared with controls (Table 2). Analysis of variance showed that only the CD26 cells were significantly different ($p < .01$).

The percentage of CD5 cells ($p < .01$) and CD4 ($p < .05 > .01$) was significantly decreased. In contrast, the percentage of CD26 cells was greatly elevated ($p < .01$), compared with controls. The CD25 cells were decreased, but not significantly, whereas CD19 cells were unchanged, compared with controls.

Autoantibodies. All autoantibodies—except antimitochrondrial— were elevated, compared with individuals from both control groups (Table 3). The odds ratio for ABB was significant (95% confidence interval [95%CI]). Also, the odds ratios for the percentage of individuals with 1 and 2 or more autoantibodies were significant (95% CI), whereas that for 3 or more was not.

Four of the 9 (44.4%) chlorpyrifos subjects were positive for antimyelin antibodies (data not shown). In comparison, 24% of the Group A control was positive.

Five subjects were tested for antithyroid antibodies. Three subjects were positive for antimicrosomal; one had antithyroglobulin, and two were negative for both autoantibodies (Table 3).

Discussion

These data demonstrate several immunologic abnormalities: (a) history of atopy and antibiotic sensitivities, (b) decreased percentage of T cells, (c) increased CD26 (TA1) cells, and (d) autoimmunity. Each abnormality will be discussed briefly.

The presence of atopy and antibiotic sensitivities suggests that the subjects in this study may be predisposed to chemical injury. For example, penicillins and penicillamine are associated with allergies and autoimmunity.[17] Although this is a small and possibly nonrepresentative sample, these observations suggest that HLA phenotypes (e.g., DR3, DR4, and DR5) and atopy may play a role in some forms of chemical hypersensitivities and should, therefore, be studied further.[17]

Given that the majority of the subjects in this study had a previous health history of hay fever and antibiotic sensitivity, it may be argued that the observed immunologic abnormalities are related to this history. Certain observations, however, contradict this argument. First, one patient did not have such a history, but developed multiple allergies and antibiotic sensitivities after exposure. Second, four of the subjects developed antibiotic sensitivities after the insecticide exposure. Third, two patients were diagnosed with either SLE or Lupus-like syndrome after their exposure. And, finally, there were nine additional individuals who were exposed to chlorpyrifos who had identical immunologic findings. Three of the nine subjects did not have prior histories of sensitivities, but have developed them subsequent to exposure (work in progress).

Table 2.—Mean Absolute Counts (ABS) of WBC, Lymphocyte, and Lymphocyte Subsets and Mean Percentage of Lymphocyte Subsets ± *SD*s for Exposed and Two Controls (Groups A and B)

	Exposed (n = 12) ABS (%)	Group A (n = 27) ABS (%)	Group B (n = 29) ABS (%)	F	p
WBC	7 011 ± 1 584	6 824 ± 1 742	6 886 ± 1 527	0.055	NS
Lymphocyte	2 845 ± 650	2 392 ± 707	2 517 ± 550	2.185	NS
CD5	1 901 ± 533	1 772 ± 576	1 906 ± 462	0.525	NS
	(66.8 ± 9.6)	(74.1 ± 7.7)	(75.6 ± 5.6)	6.349	<.01
CD4	1 336 ± 381	1 234 ± 421	1 336 ± 324	0.739	NS
	(47.9 ± 9.6)	(51.3 ± 5.9)	(53.1 ± 4.7)	3.583	<.05>.01
CD8	640 ± 258	597 ± 219	597 ± 175	0.216	NS
	(22.4 ± 6.4)	(25.2 ± 6.4)	(23.7 ± 4.5)	1.097	NS
CD4/CD8	2.3 ± 0.76	2.2 ± 0.72	2.3 ± 0.59	0.293	NS
CD19	196 ± 167	143 ± 103	202 ± 65	2.452	NS
	(7.0 ± 5.0)	(6.1 ± 4.5)	(8.0 ± 2.0)	1.751	
CD25	47.3 ± 64.4	71.2 ± 45.6	61.8 ± 42.4	1.046	NS
	(1.5 ± 1.8)	(3.3 ± 2.7)	(2.4 ± 1.6)	2.942	NS
CD26	372 ± 392	122 ± 93	52.1 ± 43	14.489	<.01
	(13 ± 13.4)	(5.1 ± 3.6)	(2.1 ± 1.8)	13.570	<.01

Table 3.—Percentage of Each Autoantibody Found in Sera of Exposed (EXP) Versus Control Groups A and B and Percentage of Individuals Who Had 1 or More, 2 or More, or 3 Autoantibodies

	EXP (n = 12) %	Group A (n = 28) %	Group B (n = 27) %	Odds ratio (b) (95% CI) EXP/A	Odds ratio (b) (95% CI) EXP/B
		Percentage of autoantibody in sera of EXP vs. control groups			
ASM	41.7	14.3	14.8	3.99 (0.62, 25.55)	3.83 (0.77, 24.54)
APC	33.3	3.6	3.7	9.7 (0.80, 117.6)	9.35 (0.77, 114.09)
ABB	58.3	7.1	3.7	14.45 (1.73, 120.8)	24.09 (2.03, 284.8)
AMIT	0	0	0	—	—
ANA	25	3.6	0	6.75 (0.52, 86.53)	*
		Percentage of individuals with ≥1, ≥2, or 3 autoantibodies			
1+	83.3	21.1	14.8	14.54 (1.9, 111.3)	21.9 (2.6, 184.9)
2+	50	7.1	3.7	10.6 (1.29, 86.9)	18.3 (1.55, 217.6)
3+	25	0	3.7	*	6.75 (0.52, 86.3)

Note: Odds ratios (95% CI) are given for exposed, compared with control groups A and B.
*Not calculated because of zero value for controls.
†Large CI as a result of small numbers per cell.

The findings for WBC, lymphocytes, and lymphocyte subsets, although not statistically significant, showed an increase in absolute numbers, compared with the control groups, Interestingly, the percentage of CD5 and CD4 cells were reduced significantly, probably resulting from the many CD26 cells. Similar observations have been reported following exposure to PBBs,[19] PCBs,[20] and aldicarb.[5] The exact nature of the T and B cell changes in this and other toxic exposures is not known. However, the changes are compatible with an increase in cortical thymic and CD10 cells and with decreased suppressor/inducer cells, all of which are reported for chlordane[3] and PCP1 exposure.

Increased expression of CD26 (TA1) cells, associated with autoimmunity, is inferred from observations on multiple schlerosis[20] and following exposure to formaldehyde,[7,8] chlordane/heptachlor,[2,3] and PCP.[1] Exposure to chlorpyrifos adds to and extends previous observations on xenobiotics. Therefore, the findings suggest that chlorpyrifos exposure is causally related to CD26 expression and autoimmunity.

It is not known at this time what the mechanisms are regarding xenobiotic exposure and subsequent immunologic abnormalities, e.g., changes in T and B cell phenotype and expression and the presence of autoimmunity. However, the observations regarding antibiotic sensitivities—both before and after exposure—point to a possible role of HLA phenotypes. In addition, as suggested by McConnachie and Zahalsky,[3] it is possible that continuous release of metabolites stored in adipose tissue may also play a role in these immunological abnormalities. This is intriguing because the chlorinated metabolites of chlorpyrifos are stored in adipose tissue.[21]

Table 4.—Summary of Autoantibodies to Thyroglobulin and Thyroid Peroxidate in the Five Subjects Tested*

Subject (age/sex)	Antithyroglobulin	Antimicrosomal
	Radioimmunoassay	
38/M	0	0
28/F	0	0
45/F	0	110
46/F	1.9	12.0
Expected	0.0–0.13 MRC	0.0–0.10 MRC
	Elisa immunoassay	
?/F	1:10	1:80
Reference	<1:20	<1:20

*Expected values are provided.

In conclusion, the presence of several different types of autoantibodies, e.g., antimyelin, antismooth muscle, antibrush boarder, and antimicrosomal, indicates that generalized tissue injury has occurred. Moreover, these identical observations have been made in additional chlorpyrifos patients (research in progress). Thus, chlorpyrifos,

as used in pesticide sprays, should be examined more closely as a probable immunotoxin.

* * * * * * * * * *

We thank Gilbert Salazar and the technical staff for their assistance. We deeply appreciate the considerate attention provided by the following physicians: David Imes, Donald L. McNeil, Albert Robbins, David Busher, Fred A. Hurst, Paula Davey, George R. Borrell, and Linwood W. Custalow.

Requests for reprints should be sent to Jack D. Thrasher, Ph.D., International Institute of Research for Chemical Hypersensitivity, 11330 Quail Creek Road, Northridge, CA 91326.

* * * * * * * * * *

References

1. McConnachie PR, Zahalsky A. Immunological consequences of exposure to pentachlorophenol. Arch Environ Health 1991; 46:249-55.
2. Broughton A, Thrasher JD, Madison RA. Chronic health effects and immunological alterations associated with exposure to pesticides. Comments Toxicol 1990; 4:59–71.
3. McConnachie PR, Zahalsky A. Immune alterations in humans exposed to the termiticide technical chlordane. Arch Environ Health 1992; 47(4):295–301.
4. Knutsen AP, Roodman ST, Evans RB, et al. Immune studies in dioxin-exposed Missouri residents: Quail Run. Bull Environ Contam Toxicol 1987; 39:481–89.
5. Fiore MC, Anderson HA, Hong R. Chronic exposure to aldicarb-contaminated groundwater and human immune function. Environ Res 1986; 41:633–45.
6. Rosenthal NE, Cameron CL. Exaggerated sensitivity to an organophosphate pesticide. Am J Psychiat 1991; 148:2.
7. Thrasher JD, Broughton A, Madison RA. Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch Environ Health 1990; 45:217–23.
8. Madison RA, Broughton A, Thrasher JD. Immunologic biomarkers associated with exposure to exothermic by-products of a urea formaldehyde spill. Environ Health Perspect (in press).
9. Broughton A, Thrasher JD. Antibodies and altered cell-mediated immunity in formaldehyde-exposed humans. Com Toxicol 1988; 2:155–70.
10. Boyuma A. Isolation of mononuclear cells and granulocytes from blood. Scand J Clin Lab Invest 1968; 21(suppl 97):77–89.
11. Englemane EG, Warnke R, Fox FI, Levy R. Studies on human lymphocyte-T antigen recognized by monoclonal antibody. PNAS, 1981; 78:791–95.
12. Fox DA, Hussey RE, Fitzgerald KA, et al. TA1, a novel 105 KD human T cell activation marker defined by a monoclonal antibody. J Immunol 1984; 133:351–54.
13. Nakamura RM, Tucker ES. Antibodies as reagent. In Henry JD (Ed.): Diagnosis and management by laboratory methods. Philadelphia, PA: WB Saunders, 1982; pp. 122–77.
14. Edgington TS, Dalessio DJ. The assessment by immunofluorescence methods of human antimyelin antibodies in man. J Immunol 1970; 105:248–55.
15. Beever K, Bradbvury J, Phillips D et al. Highly sensitive assays of autoantibodies to thyroglobulin and thyroid peroxidase. Clin Chem 1989; 35:1949.
16. Holford TR, Walter SD, Dunnet DW. Simultaneous interval estimates of the odds ratio in studies with two or more comparisons. J Clin Epidemiol 1989; 42:427–34.
17. Gleichman E, Kimel I, Purchase IFH. Immunotoxicology: suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. Arch Toxicol 1989; 63:257–73.
18. Lu YC, Wu YC. Clinical findings and immunological abnormalities in Yu-Chen patients. Environ Health Perspect 1985; 59:17–29.
19. Lee T-P, Chang K-J. Health effects of polychlorinated biphenyls. In Dean J, et al. (Eds.): Immunotoxicology and immunopharmacology. New York: Raven Press, 1985; pp. 415–22.
20. Hafler DA, Fox A, Manning ME, et al. In vivo activated lymphocytes in the peripheral blood and cerebrospinal fluid in patients with multiple sclerosis. New Engl J Med 1985; 312:1405–11.
21. Dishburger HJ, McKellar RL, Pennington JY, Rice JR. Determination of residues of chlorpyrifos, its oxygen analogue, and 2,5,6-trichloro-2-pyridinol in tissues of cattle fed chlorpyrifos. J Agricul Food Chem 1977; 25:1325–29.